NUTRIENT REMOVAL USING BIOFILM REACTOR WITH SUPPORT MEDIA

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Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in
Partial Fulfillment of the requirement for the
Degree of Bachelor Engineering in Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2009

I declare that this thesis entitled *"Nutrient Removal Using Biofilm Reactor with Support Media"* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Shafie @ Safie Bin Che Mohd Salleh and Mrs. Fauziah Binti Ibrahim

Loving brothers and sisters;
Nariah, Nor Aieda, Siti Normazura and Sallehudin

Supportive friends

For Their Love, Support and Best Wishes.

ACKNOWLEDGEMENT

In the name of Allah, the most Gracious and most Merciful.

Praise be to Allah for the blessing and guidance for me to completed this final year project.

First and foremost, I would like to thank my supervisor, Madam Norazwina Binti Zainol for her invaluable advice and contributions to this work. It is a pleasure to have an advisor being so joyful in her work.

Secondly, I would like to thank all lecturers in Faculty of Chemical Engineering and Natural Resources (FKKSA) who involved directly or indirectly in helping me to complete this project.

To all my friends and all my course mates, thanks you for helping and supporting me during the process in completing this research. I also want to express gratitude to my father, mother and my siblings for their unconditional support and encouragement throughout my studies in Universiti Malaysia Pahang (UMP).

ABSTRACT

The phosphorus removal becomes very important nowadays in order to reduced eutrophication. The aim of this study is to find the suitable loading rate for the highest phosphorus removal efficiency using biofilm reactor. Experiments are carried out in eight sequencing batch reactors (SBRs) at hydraulic retention time (HRT) of 5 d. This experiment is operated at loading rates of 5.0, 4.5, 4.0, 3.5 and 3.0 mg/L.d. The biofilm growth is increased with the increasing of loading rate according to the suspended solids readings. Loading rate 5 mg/L.d shows the highest suspended solid concentration which is 1585 mg/L at average reading. The Phosphorus removal efficiency was increasing according to the increasing of loading rate while the chemical oxygen demand (COD) removal is decreased with the increasing of loading rates. The loading rate 5.0 mg/L.d shows the highest average removal efficiency ranged from 66% to 87%. The COD removal is highest at loading rate 3.5 mg/L.d with 83% of average removal. The removal efficiency was influenced by the biofilm growth according to the suspended solid readings. The highest suspended solids reading give the highest removal efficiency. According to the Design Expert plotted, the highest predicted phosphorus removal can be achieved at loading rate 5.0 mg/L.d with 72.53% of phosphorus removal, 76.14% COD removal and 1142.85 mg/L of suspended solid (SS) concentration. The highest predicted COD removal can be achieved at loading rate (LR) 3.0 mg/L.d with 77% removal. The expected phosphorus removal is 66% and the expected suspended solid (SS) concentration is 984 mg/L. As the conclusion, the mixed culture from soil is capable of degrading phosphorus at which the effective loading rate is 5.0 mg/L.d.

ABSTRAK

Penyingkiran fosforus daripada air sisa menjadi sangat penting pada masa ini dalam mengurangkan masalah eutropikasi. Matlamat kajian ini adalah untuk mencari kadar beban yang sesuai untuk mendapatkan peratusan penyingkiran fosforus yang paling tinggi menggunakan reaktor biofilem. Eksperimen ini di jalankan di dalam lapan reaktor sesekumpul berjujukan pada masa penahanan hidraulik 5 h. Eksperimen ini dijalankan pada kadar beban 5.0, 4.5, 4.0, 3.5 dan 3.0 mg/L.h. Bacaan kepekatan pepejal terampai yang diperolehi menunjukkan peningkatan dalam pertumbuhan biofilem seiring dengan peningkatan kadar beban. Kadar beban 5 mg/L.h menunjukan bacaan purata kepekatan pepejal terampai yang paling tinggi iaitu 1585 mg/L. Kenaikan kadar beban menunjukkan peningkatan kadar penyingkiran fosforus tetapi mengurangkan kadar penyingkiran permintaan oksigen kimia. Kadar beban 5.0 mg/L.h menunjukan purata peratusan penyingkiran yang tertingi iaitu di antara 66% kepada 87%. Peratusan penyingkiran dipengaruhi oleh pertumbuhan biofilem. Bacaan pepejal terampai yang paling tinggi menunjukkan peratusan penyingkiran yang paling tinggi. Berdasarkan plot dari *Design Expert*, penyingkiran fosforus yang paling tinggi diramalkan akan dapat dicapai pada kadar beban 5.0 mg/L.h dengan 72.53% penyingkiran fosforus dan 76.14% penyingkiran permintaan oksigen kimia pada kepekatan pepejal terampai 1142.85 mg/L. Penyingkiran permintaan oksigen kimia yang paling tinggi pula diramalkan akan dapat diperolehi pada kadar beban 3 mg/L.h dengan peratusan penyingkirannya sebanyak 77%. Peratusan penyingkiran fosforus yang diramalkan pada kadar beban ini ialah 66% pada kepekatan pepejal terampai 984 mg/L. Sebagai kesimpulan, kultur campuran daripada tanah berupaya mengurai fosforus di mana kadar beban yang efektif ialah 5.0 mg/L.h.

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LIST OF ABBREVIATIONS

AIDS	-	Acquired immune deficiency syndrome
A/O	-	Anaerobic and oxic
Bio-P	-	Biological phosphorus
BOD	-	Biological oxygen demand
CISTR	-	Continuous Ideally Stirred-Tank Reactor
CSTR	-	Continuous Stirred-tank Reactor
COD	-	Chemical oxygen demand
DIN	-	Dissolved inorganic nitrogen
DIP	-	Dissolved inorganic phosphorus
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
EBPR	-	Enhanced biological phosphorus removal
EHS	-	Environmental Health and Safety
HRT	-	Hydraulic retention time
LR	-	Loading rate
N	-	Nitrogen
P	-	Phosphorus
RAS	-	Return activated sludge
rRNA	-	Ribosomal ribonucleic acid
SBR	-	Sequencing batch reactor
SS	-	Suspended solids
TEM	-	Transmission electron microscopy
WCMC	-	Weill Cornell Medical College

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CHAPTER 1

INTRODUCTION

1.1 Background

Water pollutants come from point and non-point sources. Their effects on aquatic systems largely depend on whether polluted waters are standing (lakes and ponds) or flowing (rivers). Standing systems are generally more susceptible because of slow turnover. The major water pollutants are organic nutrients, inorganic nutrients, infectious agents, toxic organics, sediment and heat. Organic nutrients come from feedlots, municipal sewage treatment plants, and industry. They promote growth of natural populations of aquatic bacteria. Bacterial decomposition of organic materials results in declines in dissolved oxygen, with dire effects on other oxygen-requiring organisms.

Two inorganic plant nutrients of major concern are nitrogen and phosphorus. They come primarily from septic tanks, barnyards, heavily fertilized crops, and sewage treatment plants, and cause excessive plant growth that clogs navigable waterways. Bacterial decay of plants in the fall result in a drop in dissolved oxygen, which may suffocate fish and other organisms.

As well known, nowadays eutrophication is one of the main problems nowadays encountered in the monitoring of the environmental water sources in the industrialized countries. It caused by the excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment (Lenntech, 1998). Eutrophication of waterways through delivery of phosphorus (P) and nitrogen (N) from farmland is an increasing problem in many countries (Haygarth and Jarvis, 1999). Eutrophication is the fertilisation of surface water by nutrients that were previously scarce. Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily (Pink and West, 2006).

This research is done to treat phosphorus from wastewater by using microorganism from soil. This study is undertaken to evaluate the effectiveness of microorganism in soil to remove phosphorus. Laboratory scale study will be conducted to determine the effects of different loading rate in the degradation of phosphorus, reduction of chemical oxygen demand (COD) and also the effects to the mixed culture growth in terms of suspended solid that exist during the experiment.

1.2 Problem Statement

Nowadays, world has become aware about the seriousness of one of the major effect of the waste discharged by the industrial activities, that is the quantity and diversity of hazardous waste. This harmful and toxic discharge will affect our aquatic system. So, it is important to control the waste contain to assure that our ecosystem are prevented from damage.

In typical wastewater treatment process, optimum dosing of the appropriate chemicals, such as phosphorus is added into the water. Phosphorus will contribute to the poor surface water quality when discharged it to the river. The excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment will cause eutrophication. The increasing of phosphor concentrations in surface waters raises the growth of phosphate-dependent organisms, such as algae (Phytoplankton). These organisms use great amounts of oxygen and prevent sunlight from entering the water. This makes the water fairly unliveable for other organisms (Lenntech, 1998).

This research is proposed because the method that will be use is quite economical since it only uses the microorganism from soil which is easy to get from our surrounding. These microorganisms are assumed can treat the phosphorus in the wastewater.

1.3 Objective of the Study

The main objectives of this research are:

1. To study the degradation of phosphorus by microorganism in soil.
2. To study the effect of different loading rate on phosphorus removal.
3. To study the effect of phosphorus concentration to the growth of mixed culture.

At the end of the study, this objective will help in understanding the used of mixed culture in phosphorus removal process which is the main concern of this study.

1.4 Scope of the Study

The scope of this study is to acclimatize the bacteria in order to treat phosphorus from wastewater and to monitoring the mixed culture growth using suspended solid test. The wastewater that contains of phosphorus in the phosphate form (PO_4^{3-}) was simulated with the appropriate nutrients for mixed culture. The experiments were conducted in biofilm reactor with different loading rate. The efficiency of the treatment for different phosphate concentration is evaluated in terms of water quality parameters; chemical oxygen demand (COD) and the removal of the initial concentration. Besides, the effect of the phosphate concentration on microorganism growth in terms of suspended solids concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Phosphorus

Phosphorus is the chemical element that has the symbol P and atomic number 15. The name comes from the Greek meaning “light” and “bearer”. A multivalent nonmetal of the nitrogen group, phosphorus is commonly found in inorganic phosphate rocks. Due to its high reactivity, phosphorus is never found as a free element in nature on Earth. One form of phosphorus (white phosphorus) emits a faint glow upon exposure to oxygen, and hence in Greek derivation, meanings "light-bearer" (Latin *Lucifer*), the planet Venus as "Morning Star". Phosphorus is a component of DNA and RNA and an essential element for all living cells. The most important commercial use of phosphorus-based chemicals is the production of fertilizers. Phosphorus compounds are also widely used in explosives, nerve agents, friction matches, fireworks, pesticides, toothpaste, and detergents. Phosphorous is a multivalent nonmetal of the nitrogen group. It is found in nature in several allotropic forms, and is an essential element for the life of organisms. There are several forms of phosphorous, called white, red and black phosphorous. Eventhough their colours are more likely to be slightly different. White phosphorous is the one manufactured industrial; it glows in the dark, is spontaneously flammable when exposed to air and is deadly poisoning. Red phosphorous can vary in colours from orange to purple, due to slight variations in its chemical structure. The third form, black phosphorous, is made under high pressure, looks like graphite and, like graphite, has the ability to conduct electricity (Lenntech, 1998).

2.1.1 Phosphorus Sources

In the natural world, phosphorous is never encountered in its pure form, but only as phosphates, which consists of a phosphorous atom bonded to four oxygen atoms. This can exists as the negatively charged phosphate ion (PO_4^{3-}), which is how it occurs in minerals, or as organophosphates in which there are organic molecules attached to one, two or three of the oxygen atoms (Lenntech, 1998).

Municipal wastewaters may contains from 5 to 20 mg/L of total phosphorous of which 1-5 mg/L is organic and the rest in inorganic. The individual contribution tend to increase, because phosphorous is one of the main constituent of synthetic detergents. The individual phosphorous contribution varies between 0.65 and 4.80 g/inhabitant per day with an average of about 2.18 g. The usual forms of phosphorus found in aqueous solutions include orthophosphate, polyphosphate and organic phosphate (Lenntech, 1998).

Phosphate rock, which is partially made of apatite (an impure tri-calcium phosphate mineral) is an important commercial source of this element. About 50 per cent of the global phosphorus reserves are in the Arab nations. Large deposits of apatite are located in China, Russia, Morocco, Florida, Idaho, Tennessee, Utah, and elsewhere (Albright and Wilson, 1956).

2.1.2 Disadvantage of Phosphorus

As well known, nowadays eutrophication is one of the main problems nowadays encountered in the monitoring of the environmental water sources in the industrialized countries. It caused by the excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment (Lenntech, 1998). Eutrophication of waterways through delivery of phosphorus (P) and nitrogen (N) from farmland is an increasing problem in many countries (Haygarth and Jarvis, 1999).

In white phosphorus form, it enters the environment through discharge of wastewater, white phosphorus ends up in surface waters near the factories that use it. White phosphorus is not likely to spread, because it reacts with oxygen fairly quickly. When phosphorus ends up in air through exhausts it will usually react with oxygen right away to be converted into less harmful particles. However, when phosphorus particles are in air they may have a protective coating that prevents chemical reactions. In water, white phosphorus is not reacting with other particles that quickly and as a result it will accumulate in the bodies of aquatic organisms. In soil, phosphorus will remain for several days before it is converted into less harmful substances. But, phosphorus may remain for a thousand years in deep soils, the bottom of rivers and lakes (Lenntech, 1998).

Phosphorus exist in phosphate form in natural world, phosphates have many effects upon organisms. The effects are mainly consequences of emissions of large quantities of phosphate into the environment due to mining and cultivating. During water purification phosphates are often not removed properly, so that they can spread over large distances when found in surface waters. The increasing phosphor concentrations in surface waters raise the growth of phosphate-dependent organisms, such as algae (Phytoplankton). These organisms use great amounts of oxygen and prevent sunlight from entering the water. This makes the water fairly unlivable for other organisms (Lenntech, 1998). The key role of this substance is particularly evident when one realizes that 1g of PO^4-P enables the development of 100g Phytoplankton. For the aerobic breakdown of these biomes, further 150g oxygen is required. In other words, the presence of 1g of PO^4-P in waterways induces a secondary overhead of 150g biochemical oxygen demand (BOD). This phenomenon is commonly known as eutrophication (Uhlmann, 1982).

Forsberg and Ryding, (1980) give the trophic status of waterways with respect to the total phosphorus concentration as; Concentration < 15µg PO^4-P/l are oligitrophic, concentrations of 15-25µg PO^4-P/l is mesotrophic and concentrations of >25µg PO^4-P/l is eutrophic. From these concentration figures, the importance of phosphate removal from wastewater becomes clear.

2.1.3 Level Measurement to Discharge Phosphorus

Several types of wastes (chemical, biological, radioactive, universal, and recyclable) are generated by a variety of laboratory, maintenance, and cleaning operations at the Weill Cornell Medical College (WCMC). Wastes must be properly managed by personnel in their work areas prior to collection for disposal. These Waste Disposal Procedures have been established as part of the WCMC Environmental Health and Safety (EHS) Program Manual to provide generators guidance in the proper management of chemical, biological, radioactive, universal, and recyclable wastes (EHS).

Many countries set 1 mg/L and 2 mg/L as the limit for total phosphorus concentration in discharge of wastewater treatment plants. One of the reasons for this low limit is that P concentrations below 0.5 mg/L have been shown to be the limiting value for Algae growth (Dryden and Stern, 1968).

For Asian country such as Malaysia, the level measurement to discharge phosphorus is 0.2 mg/L; this is based from Department of Environment in national water quality standard in Malaysia.

2.2 Mixed Culture

2.2.1 Microorganism in Sediment

The deep sea and its sediments represent the largest permanently cold environment on Earth. We incubated cultures at low temperatures (2°–10°C) in order to increase the number of psychrophilic microorganisms and to explore their potential enzyme activities in situ. We isolated and characterized organisms that are phylogenetically related to *Photobacterium, Halomonas, Shewanella,* and *Vibrio* species. All isolates are closely related (by >98% 16S rRNA similarity) to previously isolated deep-sea strains, consistent with their being from the core sample rather than contaminants. These genera are also commonly found in deep sediment studies, especially those in the Pacific (Wang *et al.,* 2004). The trend in extracellular degradative enzyme production agrees with previously published results of deep-sea sediment isolates and corresponds to available nutrient sources, suggesting a possible adaptation to this environment or competitive advantage within this ecosystem (Wang *et al.,* 2004). The one *Vibrio* sp. isolate from the sediment column (0.67 mbsf) adds to the database of other sediment-dwelling microbes isolated from deeper than 0.5 mbsf (Bale *et al.,* 1997). Further, the characterization of these isolates increases the numbers of described species for these genera and provides information on their production of cold-active enzymes of possible industrial interest.

The presence of a number of facultative anaerobic microorganisms in our cultivations suggests that the capability for anaerobic growth may allow cell survival after burial by the accumulation of the sediment column over time. However, because facultative organisms grow more rapidly aerobically, especially at low temperatures, we used aerobic cultivation to examine this population. In such an aerobic enrichment culture, it was surprising to find crenarchaeal signatures. *Crenarchaea* have been shown to be members of the seafloor community and have been detected deeper in sediment columns (Vetriani *et al.,* 1998; Bidle *et al.,* 1999). However, to our knowledge, they have not yet been reported as members of a cultivated community from marine sediment. Here we report the existence of a marine benthic *Crenarchaeon* in a bacteria-dominated enrichment culture at 10°C for more than a week. The conditions needed to prolong the existence of these *Crenarchaea* in liquid culture are being investigated.

The importance of the bacterial population with respect to enhanced biological phosphorus removal (EBPR) has been noted in a number of papers (Carucci *et al.,* 1995). Kavanaugh, (1991) found that bacteria belonging to *Aeromonas*/*Vibrio*, Coliforms, *Pseudomonas* and *Acinetobacter* were present in a continuous flow EBPR system, which was also operated to achieve biological nitrogen removal. In the same study, *Acinetobacter* which is widely reported to be responsible from phosphorus uptake only accounted for 5% of the population. This finding was supported that it was difficult to find *Acinetobacter* in well-operated fill and draw activated sludge systems (Hascoet, 1985). In a study carried out by Okada *et al*. (1992) however, *Acinetobacter* and *Pseudomonas* were the predominant species and high phosphorus removal efficiencies were obtained. *Pseudomonas* is present in fish and sediments from aquaculture in Australia (Olasumbo**,** 2007**)**.

2.2.2 Microorganism in Soil

The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. Microorganisms are found in large numbers in soil usually between one and ten million microorganisms are present per gram of soil with bacteria and fungi being the most prevalent. However, the availability of nutrients is often limiting for microbial growth in soil and most soil microorganisms may not be physiologically active in the soil at a given time.

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like carbon and nitrogen, which are required for plant growth. For example, soil microorganisms are responsible for the decomposition of the organic matter entering the soil (e.g. plant litter) and therefore in the recycling of nutrients in soil. Certain soil microorganisms such as mycorrhizal fungi can also increase the availability of mineral nutrients (e.g. phosphorus) to plants. Other soil microorganisms can increase the amount of nutrients present in the soil. For instance, nitrogen-fixing bacteria can transform nitrogen gas present in the soil atmosphere into soluble nitrogenous compounds that plant roots can utilize for growth. These microorganisms, which improve the fertility status of the soil and contribute to plant growth, have been termed 'bio-fertilizers' and are receiving increased attention for use as microbial inoculants in agriculture. Similarly, other soil microorganisms have been found to produce compounds (such as vitamins and plant hormones) that can improve plant health and contribute to higher crop yield. These microorganisms (called 'phytostimulators') are currently studied for possible use as microbial inoculants to improve crop yield (Impact, 1998).

2.2.3 Microorganism in Geyser

The ribbons of color that stream from hot springs are usually formed by a variety of bacteria. The green and orange mats that you see here live in water of varying temperatures; in essence, the colors serve as temperature indicators. Hot springs *cyanobacteria* are wonders of life at high temperatures. Some live in waters as hot as 167° F (76° C). At this temperature they are usually yellow, but become darker - orange, rust or brown - as the water cools. Between 113 °F and 131°F (45°C and 55° C), other species may appear which will modify the colors even more. Certain varieties are scientific curiosities because they are extremely specific for their environment. They may be found around the world living only in hot spring waters. Yellow or pink strands of bacteria sometimes appear in water as hot as 196° F (92° C), just below the boiling point (water boils at 199°F (93° C) at this elevation). Chemical deposits of sulphur, iron oxides, arsenic sulfide, and other substances add vivid colors to the hot springs in a few areas of the park, but not generally in the Midway and Fountain Paint Pots Basins. At Norris Geyser Basin the Echinus Geyser and Emerald Spring release acidic water. Hardy microscopic plants, like lime-green *Cyanidium* algae, thrive in these warm acid waters. *Orangish cyanobacteria* may be found in many runoff streams in Porcelain Basin. From a distance these bacteria look like rusty iron-rich mineral deposits. Amazingly, living organisms thrive even in the extreme environments of Norris' acid hot springs. These bacteria are on the cutting edge of research in the fields of medicine and criminal investigation, yielding new tools in such complex areas as AIDS research and DNA "fingerprinting" (Karen, 1995).

The acidophilic and thermophilic unicellular red alga, Cyanidium caldarium (Tilden) Geitler, is widely distributed in acidic hot springs. Observation by transmission electron microscopy (TEM) showed that algae grown in Allen's medium contained electron-dense bodies with diameters from 100 to 200 nm.

Electron dispersive x-ray analysis indicated that the electron-dense bodies contained high levels of iron, phosphorous, and oxygen; P/Fe ratios were from 1.3 to 2.0 (Mori *et al.*, 2003).

Preconditions for the development of diazotrophic cyanobacteria have been described many times for marine and freshwater ecosystems (Berman *et al.*, 1998). One of the most important preconditions is a low N to:P ratio of inorganic the availability of dissolved inorganic phosphorus (DIP) in water when dissolved inorganic nitrogen (DIN) is already exhausted (Kononen *et al.*, 1996). This precondition exists in the Baltic Sea. In comparison with the Redfield ratio, the low N: P ratio of about 8:1 in the winter surface layer generated by vertical convection is the result of denitrification and DIP release in the anoxic deep water (Hille *et al.*, 2005).

2.2.4 Microorganism Selection

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. Certain soil microorganisms such as mycorrhizal fungi can also increase the availability of mineral nutrients (e.g. phosphorus) to plants. Thus, microorganism from soil is chosen for this research because it seems to be effective way to treat phosphorus. Microorganism from soil also easy to get because can be taken from our surrounding.